GROalpha regulates human embryonic stem cell self-renewal or adoption of a neuronal fate.

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Public Summary:
Scientists are constantly seeking new and better ways to grow human embryonic stem cells. A major goal is to simplify the conditions. In the past, human embryonic stem cells have been grown on a layer of another cell type, termed “feeders.” Previously, our group showed that cells isolated from human placentas were excellent feeders. However, we wanted to eliminate the need for feeders that could be a potential source of infectious agents. Thus, we sought to identify the molecules that the feeder cells secreted that enabled the growth of human embryonic stem cells. Specifically, we compared the factors that are released in culture by placental feeders to other cells of a similar type that lack this biological activity. We were especially interested in families of molecules that are termed chemokines because they signal cells to move, or growth factors because they promote self-renewal. A broad-based analysis showed that the placental feeders, among all the molecules screened, expressed six times higher levels of the chemokine, GROα. Interestingly, human embryonic stem cells also expressed a receptor molecule that could bind this chemokine, which suggested that GROα could have important effects. Accordingly, we used this information to develop defined culture medium that contained GROα, essentially liquid food for “feeder-free” propagation of stem cells. We also grew the cells on a coating of human blood proteins. This promoted their ability to adhere, which is also required for growth. To test the utility of this formulation, we grew the cells for many generations in these new conditions. We compared their properties to cells that were grown under standard conditions with feeders. The results were very favorable. The stem cells that were grown in this new way were normal. The endpoints that we examined included a property termed apical-basal polarity. In other words, stem cells have a distinct top and bottom as well as tight connections to one another. Indeed, cells that were cultured in the new medium were polarized. They were also normal with regard to the number of chromosomes they carried, their fingerprint in terms of markers of plasticity and their ability to form precursors of all the major cell types in the body. However, this was only true if the cells were propagated as small aggregates rather than individually. Single cells cultured under these conditions became non-polarized and differentiated into early-stage neurons as determined by their expression of sets of markers that define this lineage. We drew several conclusions from this study. In the human embryonic stem cell system, polarity is associated with an undifferentiated (tabula rasa) state. Loss of defined apical and basal surfaces is associated with neuronal differentiation, which shows the propensity of human embryonic stem cells for form this cell type. Finally, our work also has practical implications with regard to inexpensive methods that can be used to culture stem cells without other cell types in medium that is free of animal products, highly desirable for preventing their contamination with infectious agents.

Scientific Abstract:
Previously we reported that feeders formed from human placental fibroblasts (hPFs) support derivation and long-term self-renewal of human embryonic stem cells (hESCs) under serum-free conditions. Here, we show, using antibody array and ELISA platforms, that hPFs secrete approximately 6-fold higher amounts of the CXC-type chemokine, GROalpha, than IMR 90, a human lung fibroblast line, which does not support hESC growth. Furthermore, immunocytochemistry and immunoblot approaches revealed that hESCs express CXCR, a GROalpha receptor. We used this information to develop defined culture medium for feeder-free propagation of hESCs in an undifferentiated state. Cells passaged as small aggregates and maintained in the GROalpha-containing medium had a normal karyotype, expressed pluripotency markers, and exhibited apical-basal polarity, i.e., had the defining features of pluripotent hESCs. They also differentiated into the three primary (embryonic) germ layers and formed teratomas in immunocompromised mice. hESCs cultured as single cells in the GROalpha-containing medium also had a normal karyotype, but they downregulated markers of pluripotency, lost apical-basal polarity, and expressed markers that are indicative of the early stages of neuronal differentiation-betaIII tubulin, vimentin, radial glial protein, and nestin. These data support our hypothesis that establishing and maintaining cell polarity is

essential for the long-term propagation of hESCs in an undifferentiated state and that disruption of cell-cell contacts can trigger adoption of a neuronal fate.
